SPATIAL POPULATION DYNAMICS AND OVERWINTERING BIOLOGY OF THE GLASSY-WINGED SHARPSHOOTER IN CALIFORNIA'S SAN JOAQUIN VALLEY

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ABSTRACT
The purpose of this project is to define specific environmental constraints that influence glassy-winged sharpshooter (GWSS) population dynamics and overwintering success. Experiments were conducted to determine effects of constant temperatures on the survival of GWSS adults for various exposure times under three different conditions: water-only, no water or host plant, and host plant. When only provided water, adults survived the longest (16.3 ± 1.8 days) at 15°C, with the shortest longevities at 0 and 40°C (1.5 ± 0.1 and 2.5 ± 0.3 days, respectively). Overall, the longevity patterns implied that lack of a suitable host plant would result in greater reductions in survival at higher temperatures (e.g., ≥ 25°C). When adults were provided with a preferred host plant ('Frost Eureka' lemon), percent adult survival was significantly influenced by temperature and exposure time with a significant interaction between time and treatment. Unlike the initial study where only water was provided, adult survival decreased drastically at low temperatures (0-10°C), while survival between 15-30°C averaged > 68%. Findings suggest that mortality at low temperatures could result from starvation or lack of feeding, and the critical threshold temperature required for ingestion lies between 10-15°C. In a third experiment comparing host plant presence and absence, 100% mortality occurred at 3, 21, 24 days exposure at 0, 5, and 10°C, respectively. This implies that GWSS adults cannot feed on a host plant at low temperatures (0-10°C), and further suggests that the threshold temperature for feeding falls between 10 and 15 °C. Results from these experiments will be coupled with climatological data to help to spatially define where GWSS can be expected to persist in the agricultural landscape and identify where continued management efforts can be directed to limit introductions into currently non-infested areas.

INTRODUCTION
Climate appears to play a significant role in the geographic distribution of diseases caused by *Xylella fastidiosa* (*Xf*) in California and throughout the southeastern United States (Purcell 1997). Similarly, populations of glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, in the southeastern United States appear to be constrained by climatic factors that limit the pest's establishment and persistence (Pollard and Kaloostian 1961, Hoddle 2004). Presently, limited information exists on the overwintering biology and ecology of GWSS in the San Joaquin Valley of California. A conclusion emerging is that GWSS may be limited by certain temperature thresholds at, or below which feeding may be discontinued and overwintering survivorship reduced. In turn, we are conducting experiments to carefully determine the thresholds below which feeding stops and to further determine the critical duration of time spent in this non-feeding state which may result in increased mortality. The results below and future experiments will advance our ability to define the specific environmental constraints that influence GWSS population dynamics and overwintering success by increasing our present understanding of the overwintering requirements of GWSS with a focus on critical environmental and host species factors that may limit population distribution in the Central Valley of California.

OBJECTIVES
1. Identify the critical environmental constraints that influence the spatial population dynamics and overwintering success of GWSS in California's Central Valley.
2. Characterize the impact of host plant species succession on the overwintering survivorship of GWSS populations that constrain the insect's ability to become established and persist throughout the San Joaquin Valley.

RESULTS
Objective 1:
(1) Effects of temperature on the survival of GWSS adults
Experiments were conducted to determine effects of constant temperatures on the survival of GWSS adults for various exposure times under three different conditions: water-only, no water or host plant, and access to host plant. Laboratory-reared young adults were transferred from a field station of the California Department of Food Agriculture (CDFA), Arvin,

California, and maintained at the GWSS Experimental Laboratory on the campus of California State University Fresno (CSUF). Insects were about 2-weeks old when initially used in experiments.

Adult longevity when provided water only
An experiment was conducted to determine temperature effects on GWSS adult longevity with access only to water. Adults were sexed and individually placed in clear plastic tubes (33 ml) provisioned with moist cotton balls to allow constant access to water. Insects were held under the following temperature regimes: 0, 5, 15, 20, 25, 30, and 40 (± 1) °C. Insect survival was monitored daily. Longevity of GWSS adults was significantly influenced by temperature (F = 18.99; df = 7; P < 0.001) (Figure 1A). However, there was no significant effect relative to sex or interaction between sex and temperature. Because no significant difference between sexes was found, data for both sexes were pooled to compare the means among temperature treatments (Figure 1B). At 15°C, adults survived the longest (16.3 ± 1.8 days). The shortest longevities were at 0 and 40°C (i.e., 1.5 ± 0.1 and 2.5 ± 0.3 days, respectively). Overall, the longevity patterns imply that lack of a suitable host plant would result in greater reductions in survival at higher temperatures (≥ 25°C), where insects feed at higher rates due to an elevated metabolism. In comparison, adult longevities at low temperature (5-15°C) could result from both reduced feeding and a reduced metabolism. Because adults were prevented from feeding on suitable host plants, further experiments using suitable host plants were conducted to determine temperature impacts on survival that may caused by reduced feeding (see results below).

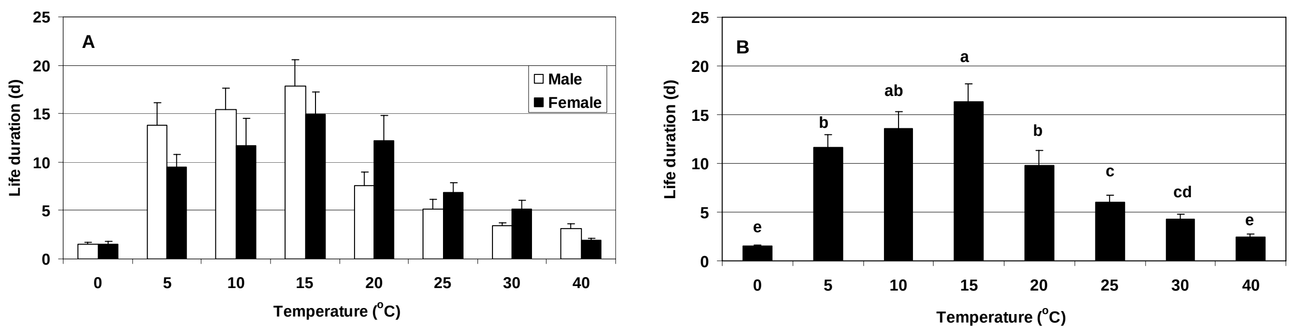

Figure1. GWSS adult longevity (days)(mean ± SEM) when only provided access to a water source: (A) males and females separately (B) data pooled for both males and females. Values followed by different letters indicates significantly different mean values among treatments (SNK test, P < 0.05).

Quantitative models were developed to describe the survivorship of GWSS in relation to the constant temperatures and exposure duration. Percentages of surviving adults were calculated at each time interval and the time to 50% mortality at each temperature was estimated by fitting the survivorship curve at each temperature to a sigmoid (polynomial) function. The survivorship curves illustrate a typical type III curve (Figure 2A), describing high initial loss followed by a period of much lower, relatively constant mortality (Pearl, 1928). Because the estimated curves were similar regardless of temperature conditions, the temperature-independent survivorship curve at normalized time (days/ days to 50% mortality) was also well described by the sigmoid function (Figure 2B; r^2 = 0.984). The estimated times to 50% mortality were 0.6, 9.2, 9.6, 13.3, 6.1, 3.8, 2.8, and 1.5 exposure days at 0, 5, 10, 15, 20, 25, 30, and 40°C, respectively. The same equation was also used to describe a temperature-independent survivorship curve at the normalized time, which was acquired by dividing the exposure time by the "time to 50% mortality" at each temperature. The relationship between temperature and the estimated time to 50% mortality was described by an extreme-value function. The model estimated that the longest time (12 days) to 50% mortality occurs at 11.1°C (Figure 2C). The skewed bell shape of the time to 50% mortality indicates that the survival of this species was more seriously impaired by the exposure to high temperature in the given condition where this species only have access to a water source.

Adult longevity when provided with host plant
Temperature effects on adult GWSS longevity were determined for individuals provided a preferred overwintering host plant. Adults were sexed and placed in a clear plastic cylinder (60 cm X 15 cm diam.) provisioned with a 'Frost Eureka' lemon plant, *Citrus limon*. Ten adults (males and females) were separately placed within cylinders and held at the following temperature regimes: 0, 5, 10, 15, 20, 30, and 40°C. The numbers of surviving adults were routinely monitored up to 21 days. Repeated measures ANOVA revealed that adult percent survival was significantly influenced by temperature (F = 70.93; df = 6; P < 0.001) and exposure time (F =133.03; df = 5; P < 0.001), with a significant interaction between time and treatment (F = 8.94; df = 30; P < 0.001) (Fig. 3). However, there was no significant effect of sex at any observation time (P > 0.05). Regardless of sex, 100% mortality occurred at 7 days exposure at 0°C. At 21 days exposure, mortality was higher than 80% at 0, 5, 10, and 40°C. Unlike the initial study where only water was provided, adult survival decreased drastically at low

temperatures (0-10°C), while survival between 15-30°C remained > 68%. As an indicator of feeding activity, production of xylem excreta was not observed at temperatures ≤ 10°C, where most adults were found on the soil surface rather than on the plant stem or leaves. These findings suggest that mortality at low temperatures could result from starvation (or lack of feeding), and the critical threshold temperature lies between 10-15°C. Mortality at 40°C probably results from heat stress on the insect and / or possible plant deterioration caused by the highly active stylet penetration on plant stems, although no visible plant stress symptoms were observed.

Comparison of survival between host and no-host conditions

Because rapid mortality was observed at low temperatures, where xylem excretion did not occur, we hypothesized that lack of feeding was a major mortality factor. A third experiment was conducted to determine whether the presence of a host plant was a critical factor at certain temperatures, with a reasonable expectation that survival would not be different between host present and host absent conditions at temperatures where feeding does not occur. Because sex was not a significant factor in previous experiments, 10 GWSS adults (males and females combined) were observed when provided a lemon plant and denied a lemon plant. The numbers of surviving adults were routinely monitored until 100% mortality was obtained in all replications. Repeated measures ANOVA revealed that percent adult survival was significantly different between treatments at 20, 30, and 40°C ($P < 0.001$). However, no significant treatment effects were observed at 0, 5, and 10°C ($P > 0.05$). In all treatments, exposure time significantly affected survival ($P < 0.0001$). Under both host and no-host conditions, 100% mortality occurred at 3, 21, and 24 days exposure at 0, 5, and 10°C, respectively. This implies that GWSS adults cannot feed on the host plant at low temperatures (0-10°C), and the threshold temperature for feeding falls between 10 and 15 °C. This is consistent with the results of the previous experiment. Notably, host plant availability was a highly critical factor for survival at high temperatures ≥ 20°C, with 100% mortality observed at 7, 3, and 2 days at 20, 30, and 40°C, respectively. In the presence of a host plant, >70% of adults survived 7 weeks exposure time at 20 and 30°C. Currently, we are completing this trial, and further analysis will be conducted on the resulting data.

(2) Effects of temperature on the feeding of GWSS adults

Laboratory experiments are underway to determine temperature effects on xylem excretia production by GWSS adults using a Parafilm sachet method (Pathak et al. 1982). Young GWSS adults (ca. 2 wk old) were individually confined inside a Parafilm sachet (7.5 x 6.5 cm) attached to the side of host plant ('Frost Eureka' lemon). Insects were held at 0, 5, 15, 20, 25, 30, and 40 (± 1)°C. Following 48-hr feeding, xylem excreta production (mg) was determined. At ≤ 15°C, no adults produced xylem excreta during the 48 hour period. The largest amount of xylem excreta production (2,200 mg) was at 40°C. We are currently replicating treatments for proper statistical analysis. After analysis, we will estimate the threshold temperature for production of xylem excreta of GWSS adults.

Objective 2

We encountered one challenge due to the legal restriction on maintaining live, caged GWSS adults in the field in quarantined areas in the San Joaquin Valley. Because of this, we plan to conduct the work in the infested areas of Kern County, where other related work has been conducted by our labs. Seasonal population dynamics of GWSS will be monitored on selected host plants placed in different micro-climatic areas of Kern County. In these experiments, we will

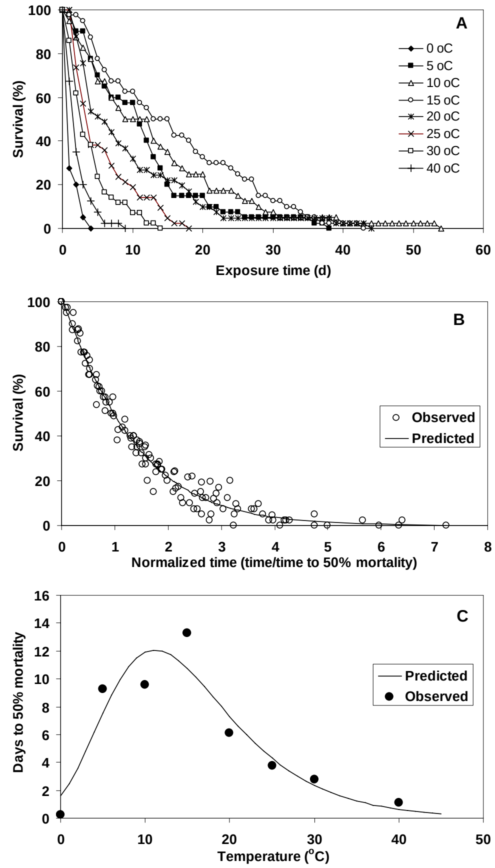

Figure 2. Survivorship curve at constant temperatures (A); the survivorship curve at normalized exposure time (days ÷days to 50% mortality) (B); and temperature-dependent model for the time to 50% mortality (C). Adults had access to water only (i.e. from moist cotton) for feeding.

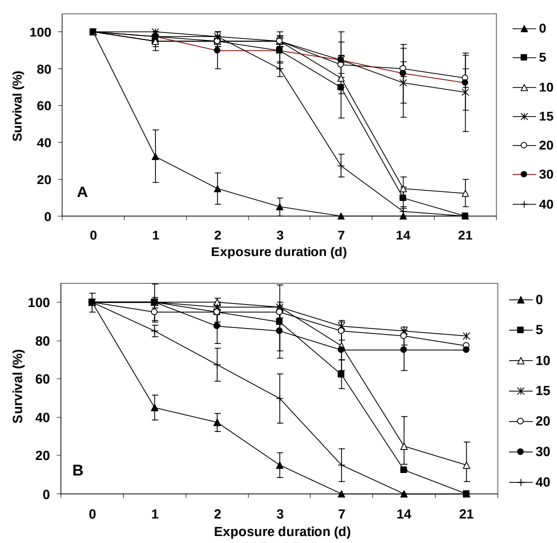

Figure 3. Percent survival (mean ± SEM) of GWSS adults provided a host plant ('Frost Eureka' lemon) at constant temperatures during 21 days exposure time: (A) males and (B) females.

examine GWSS survivorship in caged experiments on selected host plant species. In each cage, fifty 2nd generation GWSS adults, nearing reproductive diapause in the fall season, will be collected from natural infestations and released onto caged plants. Insects will be introduced onto potted plants placed in cages and populations monitored monthly throughout the winter period and in the subsequent spring. At each location, four caged replicates of host plant species including the plant species navel orange, grape, and peach will be evaluated individually and in combination. A detailed record of adult GWSS feeding and resting preference will be maintained from November 2005 to March 2006.

CONCLUSIONS

Findings from the survival tests clearly indicate that the survival and feeding activity GWSS adult is significantly influenced by temperature and exposure duration. In particular, low temperatures caused rapid mortality. Availability of feeding was a critical factor for survival at high temperatures ($\geq 20\ ^oC$). This project has a high probability of success in terms of generating significant new information regarding the thermo-biology of GWSS in California. Models generated from the experiments on survival and feeding will allow for the spatial estimation of overwintering success of GWSS.

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